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PARTITION COEFFICIENTS OF HYDROPHOBIC CONTAMINANTS IN NATURAL WATER, POREWATER, AND ELUTRIATES OBTAINED FROM DOSED SEDIMENT: A COMPARISON OF METHODOLOGIES

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ABSTRACT

Partitioning of organic contaminants in elutriates, porewater, and lake water was compared using four methods: XAD-4 resin columns, equilibrium dialysis, and centrifugation and filtration with subsequent C-18 reverse phase column separation. In addition, possible changes in partitioning with sediment aging and during bioaccumulation assays were examined. Centrifugation with C-18 separation produced the most consistent partition coefficients. Partition coefficients from filtration and centrifugation methods compared favorably with previously published data. Contaminant partitioning did not change during 96-h exposures in bioassays, and partitioning of contaminants in porewater and elutriates did not follow a linear trend with the amount of sediment aging or manipulation. The contaminant partitioning apparently depends on characteristics of the contaminant as well as contaminant-associated media composition.

INTRODUCTION

Toxicokinetic models have been used to predict contaminant exposure to organisms for both aqueous and sediment bioassays (Landrum *et al.* 1992 *a*). One such frequently used first-order rate coefficient model determines the contaminant flux into an animal as dependent on the contaminant concentration in a source compartment, typically expressed as (uptake clearance x source compartment concentration) - (animal elimination x animal concentration) (Rand and Petrocelli 1985). For sediment exposures, porewater has been suggested as the source compartment (Adams 1987, Kemp and Swartz 1988, U. S. EPA 1989). Following this assumption, numerous bioassays have utilized aqueous test fractions, either of porewater obtained from whole sediment or of elutriates (extracts of whole sediment originally intended to mimic suspension events associated with dredging operations; see Giesy and Hoke 1989 for a review). To account for the actual exposures in assays that use these aqueous test fractions, an accurate assessment of the bioavailable form of contaminant is necessary. Current theory suggests that the freely dissolved contaminant is the only form available to an organism residing in the aquatic environment for accumulation (Landrum *et al.* 1985, Kukkonen *et al.* 1990). The remainder that is bound to organic material, colloids, and other microparticulate material is unavailable over relatively short exposures (*e. g.*, hours; Voice *et al.* 1983, Chin and Gschwend 1992). If the organism is a filter feeder, some of the material on small particles or colloids may well be available through ingestion. Bound and freely dissolved phases have been measured through a variety of methods, including equilibrium dialysis (Carter and Suffet 1982, Kukkonen *et al.* 1990), C-18 reverse

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phase separation (Landrum *et al.* 1984, Maaret *et al.* 1992), water solubility enhancement (Chiou *et al.* 1986), and flourescence techniques (only suitable for fluorescent compounds; Roemelt and Setiz 1982, Schlautman and Morgan 1993). Although these methods may work well for organic contaminants at relatively high concentrations, they may not be as efficient when applied to hydrophobic organics in nanomolar quantities.

Partitioning can be quite variable in both porewater and natural waters for a single contaminant, even when corrected for the amount of organic carbon in the system (Landrum *et al.* 1985, 1987). Variation in the composition and quantity of the organic material present and the extent of laboratory manipulation involved in obtaining porewater or in dosing natural water may be responsible for the observed variability in partitioning. Further, the relative amounts and/or composition of bound and freely dissolved fractions may change during the course of a bioassay, due to the behavior of the bioassay organisms or as a result of bioassay conditions (*i. e.,* feeding). If changes in contaminant bioavailability occur and are not considered in the bioassay or toxicokinetic model, the rate of contaminant flux and thus the potential for bioaccumulation and subsequent effects may be inappropriately estimated or misunderstood.

This study examined the partitioning of organic contaminants in elutriates and porewater obtained from laboratory-dosed whole sediment and laboratory-dosed lake water. We compared partition coefficients generated from four methods to determine which methods were most suitable for estimating the bioavailable, "freely dissolved" fraction of contaminant. In addition, we wanted to determine any differences in partitioning resulting from sediment aging and manipulation, as well as any differences that may occur over various exposure intervals in a typical bioaccumulation assay.

MATERIALS AND METHODS

A. Chemicals

The compounds studied included ^{14}C-radiolabeled *trans*-chlordane (13.7 mCi/mmol, Velsicol Chemical Co., Memphis, TN), [^{14}C]-endrin (8.4 mCi/mmol, Sigma Chemical Company, St. Louis, MO), [^{3}H]-benzo(*a*)pyrene (BaP, 40.0 Ci/mmol, Sigma Chemical Co.; 69.0 Ci/mmol, Amersham Ltd., Amersham, UK), and [^{3}H]-pyrene (25.2 Ci/mmol, Chemsyn Science Laboratories, Lenexa, KS). All compounds were dissolved in an acetone carrier. Compound radiopurity was greater than 97% for all compounds prior to use, as determined by thin layer chromatography, using either benzene:ethyl acetate (3:1, v:v, endrin and *trans*-chlordane) or hexane:benzene (8:2, v:v, BaP and pyrene) and liquid scintillation counting (LSC). Analytical procedures were performed under gold fluorescent light ($\lambda \geq 500$ nm) to minimize the photodegradation of the polycyclic aromatic hydrocarbons (PAHs).

B. Sediment Dosing and Manipulation

Sediment was collected at a 45 m depth in Lake Michigan by Ponar grab approximately 8 km off the coast of Grand Haven, MI. The sediment was passed through a 1-mm sieve to remove debris and indigenous organisms. A sediment-water slurry was made by diluting wet sediment with Lake Michigan water in a 1:4 sediment to water ratio (w/v). Radiolabeled chemicals were added to the slurry drop by drop in a minimal amount of acetone carrier (<1 ml per liter wet sediment) while being stirred on a mechanical stirrer at room temperature for 4 h. Three sediments were prepared in the following combinations and range concentrations: BaP/*trans*-chlordane (BaP = 0.39 ng/g dry weight, *trans*-chlordane = 1.66 μg/g), pyrene/endrin (pyrene = 0.87 ng/g, endrin = 0.53 μg/g), and pyrene/*trans*-chlordane (pyrene = 0.64 ng/g, *trans*-chlordane = 1.74 μg/g), where one compound was ^{3}H labeled and the other was ^{14}C labeled. One sediment was dosed only with pyrene (0.76 ng/g dry weight). After stirring, sediment slurries were allowed to settle at 4°C for 48 h. Subsequently the overlying water was decanted, and the sediment was washed with another four volumes of lake water by stirring for another 4 h and settling at 4°C for 48 to 96 h. The overlying water was again decanted, and portions of the sediment were either used to prepare elutriates or centrifuged to obtain porewater for the various partitioning procedures.

C. Elutriate Preparation

A 1:4 ratio (sediment:water, v:v) of prepared dosed sediment was used to prepare elutriates (U. S. EPA/CE 1990). The sediment/water mixtures were placed into 250-ml Teflon centrifuge bottles. The bottles were placed on a laboratory rotator at 200 rpm for 30 min, then were allowed to settle for 1 h. The unsettled portions were decanted into clean Teflon centrifuge bottles and were centrifuged at 2000*g* at 10°C for 30 min. The elutriate fractions were decanted and stored for no longer than 24 h at 10°C before use in partitioning studies. Contaminant concentrations in elutriates ranged from 0.24 pg/ml (pyrene) to 54.8 ng/ml (*trans*-chlordane).

D. Porewater Preparation

Dosed prepared sediment was placed into 250-ml stainless steel centrifuge bottles and centrifuged at 4000*g* for 30 min at 10°C. Supernatant from the bottles was decanted into 250-ml Teflon centrifuge bottles and was spun at 2000*g* for 30 min at 10°C. The supernatant from all bottles was decanted and stored for no longer than 24 h at 10°C before use in partitioning studies. Contaminant concentrations in porewater ranged from 0.67 pg/ml (BaP) to 16.24 ng/ml (*trans*-chlordane).

E. Dosed Lake Water Preparation

Lake Michigan surface water was collected about 1 m below the surface and stored at 4°C until used. Quantities of lake water were filtered through glass fiber filters (Gelman type A/E, Gelman Sciences, Ann Arbor, MI), then through 0.45-μm polycarbonate filters (Nuclepore Corp., Pleasanton, CA) to remove particles. Containers of lake water were placed on a laboratory stirrer after the addition of radiolabeled compounds in acetone carrier. Compounds were added with the same compound combinations for the dosed sediments in concentrations ranging from 0.15 pg/ml (pyrene) to 37.12 ng/ml (*trans*-chlordane). Mixtures were stirred at room temperature for at least 1 h and were stored overnight in the dark at 10°C before use in partitioning studies.

F. Organic Carbon Measurements

Organic carbon in lake water, porewater, and elutriate exists in particulate, colloidal, and dissolved forms. Both filtration and centrifugation can remove some of this, depending on the filter size and centrifugal force employed. However, we chose to define all organic carbon in whole porewater, elutriate, and lake water samples as TOC, since a definite measure of organic carbon associated with colloids and microparticulates that may have remained after isolation and pre-treatment processing (*i. e.*, filtration and centrifugation) could not be determined. Aqueous measures of total organic carbon (TOC) from elutriate, porewater, and dosed lakewater were performed on an Oceanography International® carbon analyzer after persulfate digestion (Golterman *et al.* 1978). Duplicate analyses yielded differences of 1 - 10%. Replicate samples of prepared lake water, elutriate, or porewater were taken prior to each method evaluation for determination of TOC in the total aqueous material.

G. Sampling and Chemical Analysis

Aqueous samples for compound concentrations were removed in 2-ml aliquots and placed directly into 12 ml scintillation cocktail (Research Products International 3a70b, Mt. Prospect, IL). Radioactivity was determined via LSC on an LKB 1217 liquid scintillation counter. The data were corrected for quench using the external standards ratio method after correcting for background.

H. Method Evaluation

Four methods were used to determine partition coefficients. The following compounds were used to compare filtration and centrifugation methods: [^{3}H]-BaP, [^{3}H]-pyrene, [^{14}C]-*trans*-chlordane, and [^{14}C]-endrin. In addition, lake water and elutriate containing dual labeled [^{14}C]-*trans*-chlordane and [^{3}H]-BaP were used to evaluate the XAD resin methods, while elutriate from [^{3}H]-BaP dosed sediment was used in the dialysis method.

XAD Resin Method The polymeric resin XAD-4 was used to evaluate the separation and recovery of the compounds because it incorporates the properties of high surface area, porosity, and hydrophobicity (Landrum and Giesy 1981). Two methods used XAD-4. In one method, champagne columns 1.5 cm diameter x 25 cm long were plugged with glass wool and packed with 25 $\pm$ 2 ml of wet precleaned Amberlite XAD-4 resin (Rohm and Haas, Philadelphia, PA). After pre-conditioning the columns with 5 ml deionized water, 10-ml replicate samples of elutriate or dosed lakewater were percolated through the columns. After percolation, the columns were rinsed with 5 ml deionized water. The filtrate was collected and a sample was taken for LSC. This fraction was defined as the bound fraction. The columns were sequentially eluted with 10 ml acetone. Samples of the eluted fraction, defined as the freely dissolved fraction, were taken for LSC. The percent of bound compounds was calculated as the activity of compounds eluted from the columns divided by the activity of compound present in the sample prior to percolation through the column. Mass balance was calculated as the total amount of compound percolating through and eluted from the columns divided by the amount of compound present in the sample prior to percolation through the columns.

In an alternate, XAD-4 "stirred" method, 0.3 g precleaned XAD-4 resin was mixed with 10 ml of elutriate in 20-ml glass scintillation vials. The vials were manually shaken for 60 s, then left to settle. Two ml of supernatant was removed and activity determined via LSC. The remaining resin was removed by vacuum filtration through glass fiber filters (type GF/C, Whatman Ltd., Maidstone, England). The filter and resin were dried in a 90°C oven for 20 min, then placed into scintillation cocktail for analysis via LSC. Freely dissolved and bound fractions were determined from resin/filter and supernatant respectively, as described for the "column" method.

Equilibrium Dialysis The procedure for dialysis followed the methods of Carter and Suffet (1982). Distilled water was dosed with radiolabeled contaminant and mixed for 2 h in the dark at room temperature. Contaminants were directly spiked into distilled water to hasten equilibration of contaminant between elutriate (inside membrane) and the surrounding water outside the dialysis membrane. Two hundred ml of dosed water was dispensed into 250-ml brown bottles. Sodium azide (0.002%) was added to inhibit microbial growth. Nine cm lengths of 10-mm diameter dialysis tubing (Spectra/Por® cellulose ester membranes, Spectrum Medical Industries, Los Angeles, CA) were soaked in deionized water to remove the sodium azide preservative. The lengths were filled with 4 ml prepared elutriate and secured with clamps. Each dialysis bag was placed into one of the prepared brown bottles and was sealed with a Teflon-lined cap. Bottles were placed on a laboratory rotator at 100 rpm at room temperature. Controls consisted of dialysis bags filled with 4 ml distilled water instead of the elutriate. Three replicate bottles and one control were removed from the rotator at 48, 99, and 170 hours. Replicate 2 ml samples were collected from both dialysis bags and the bulk water and analyzed via LSC.

Freely dissolved contaminant was calculated from the bulk water activity (outside the membrane), while the difference between activities inside and outside the membrane was defined as the bound fraction. Mass balance was determined as the amount of chemical activity from the sum of concentrations inside, outside, and from a hexane rinse of the bottles and membranes that corrected for sorption to surfaces divided by the total activity of dosed water originally added to the bottles.

Filtration Method The C-18 reverse-phase/filtration method followed a modified procedure described by Eadie *et al.* (1990). Replicate samples of prepared lake water, elutriate, or porewater were analyzed for total contaminant activity via LSC. Fifteen-ml aliquots of the aqueous solutions were then filtered through two 25-mm glass fiber filters, using a stainless steel filter support. The top filter collected particulate matter, while the bottom filter was presumed to adsorb an equal amount of dissolved organic material. Each filter was placed into a scintillation vial with 12 ml scintillation cocktail and sonicated for 30 s, using a high intensity laboratory sonicator. Filters were counted to correct for the particulate and dissolved fractions of contaminant(s). The filtrate was sampled for contaminant activity (defined as the particulate-free fraction), and 10 ml was passed through a Waters® C-18 reverse phase Sep-Pak column (Millipore Corp., Milford, MA) that had been pre-rinsed with 5 ml of filtrate. Samples of this filtrate, defined as the bound fraction, were analyzed for contaminant activity via LSC.

The bound contaminant fraction was calculated as

$$\frac{\text{top filter activity - (2 x bottom filter activity)}}{\text{\# ml filtered}} + \text{activity of C-18 filtrate,} \tag{1}$$

where activity = dpm/ml. The freely dissolved fraction was calculated as

$$\text{filtrate activity after glass fiber filters - filtrate activity after C-18 column} + \frac{\text{(2 x bottom filter activity)}}{\text{\# ml filtered}}. \tag{2}$$

Percent of bound and freely dissolved contaminants was calculated as the activities of either freely dissolved or bound phases divided by the total activity in the sample determined prior to filtration.

Centrifugation Method Samples of prepared lakewater, elutriate, or porewater were analyzed for compound activity via LSC. Forty ml of the aqueous phases were placed in 50-ml stainless steel centrifuge tubes and were centrifuged at 20,000*g* for 30 min at 10°C. Supernatant was sampled for TOC and 10 ml were passed through a C-18 Sep Pak column. The bound fraction of contaminant in the centrifuged sample was defined as the activity of contaminant in the fraction that passed through the column. The freely dissolved fraction was calculated by subtracting the bound fraction activity from the total activity found in the supernatant. Mass balance calculations were made by dividing the activity of supernatant fractions, centrifuged pellet, and acetone rinse of the centrifuge tubes by the activity of the contaminant in the samples prior to centrifugation.

I. Calculation of Partition Coefficients

Partition coefficients, defined as K_p values, were corrected for aqueous TOC. All aqueous phases were sampled for TOC determination before partitioning manipulation with the exception of the centrifugation method, where TOC was sampled from the supernatant after centrifugation at 20,000 *g*. Partition coefficients were calculated as

$$\frac{\text{activity of bound contaminant (ng/ml) / TOC (mg carbon/ml)}}{\text{activity of freely dissolved contaminant (ng/ml)}}. \tag{3}$$

J. Sediment Manipulation and Aging

Partitioning of contaminants in porewater and elutriates generated from four sediments dosed with BaP/*trans*-chlordane, pyrene/endrin, and pyrene (2 separate batches) was examined using the centrifugation method. The sediment had previously been dosed and used in bioassays that utilized whole sediment exposures. After the exposures, bioassay organisms were removed from the sediment. Sediment from all exposure containers was combined, mixed, and placed in the dark at 4°C for various aging periods before partitioning was determined. After partitioning studies were performed on porewater and elutriates produced from one of the aged sediments (pyrene/endrin), the sediment was again recovered and stored in the dark at 4°C. One week later, porewater and elutriates were again generated from the sediment, and partitioning of the contaminants was examined.

K. Bioassay Procedures

Samples of dosed lake water, elutriate, and porewater were prepared and used in bioaccumulation assays. Two fourth instar *Chironomus riparius* larvae were subjected to 20 ml of the aqueous media in static exposures at 10°C. At the completion of predetermined exposure intervals (one-, six-, 24-, 48-, and 72-h), samples were analyzed for TOC and amounts of bound and freely dissolved contaminants using the filtration method described above. Partition coefficients were calculated and used to evaluate any changes in partitioning over the course of the assay.

L. Statistics

Two-way analysis of variance was used to test for overall significant differences in partitioning among aqueous phases and methodologies (SAS® 1988). The amount of bound contaminant was determined significantly different among the test categories at the 0.05 probability level. Scheffe's multiple range test was used for post-hoc multiple comparisons within categories. Differences between two means were compared by using Student's *t* tests. Differences were considered statistically significant when $p < 0.05$. Linear regression and correlation coefficients were used to evaluate differences in partitioning after sediment manipulation (Data Desk® 1989).

RESULTS

A. Methods Evaluation

Equilibrium Dialysis Recovery of the compounds tested using dialysis was low, where only 11 - 14% of total BaP activity could be found in bound and freely dissolved fractions after 170 h. Mean BaP concentrations inside and outside dialysis membranes were 0.34 ± 0.02 pg/ml and 0.13 ± 0.01 pg/ml, respectively, after 170 h. The percent of bound BaP in elutriates was much lower when measured by dialysis compared to the XAD and centrifugation methods, but was comparable to the filtration method (Table 1).

Dialysis was also attempted with elutriates in water dosed with *trans*-chlordane. However, even with intially high activities present in the water, activities from inside the dialysis bags after 170 h were close to background, and partition coefficients could not be evaluated. The loss of activity over the time course of the study was accounted for by sorption of compounds to glass walls of the bottles, plastic surfaces of the dialysis bag clamps, and the membranes themselves.

XAD-4 Resin A wide range of total contaminant recovery was seen with the column method (54.3 - 103.2% for BaP; 45.9 - 90.4% for *trans*-chlordane), while recovery was greater for the stirred method (97.6 - 100.0% for BaP; 85.6 - 101.2% for *trans*-chlordane). However, the transfer, filtration, and drying of the resin in the stirred method proved to be much more time consuming than column method procedures. Partition coefficients produced from the column method were more variable in BaP-contaminated elutriates than for dialysis, centrifugation, or filtration methods (Table 1).

Filtration The percent of BaP binding from the filtration method was lower than that obtained via XAD or centrifugation (Table 1). Percent of *trans*-chlordane binding was not significantly different between XAD and filtration methods (Table 2). Porewater bound the greatest percentage of BaP and *trans*-chlordane while lake water bound the least, even after adjusting for TOC, as reflected in K_p values (Tables 1 and 2). Conversely, most binding of endrin occurred in lakewater (69.51%), while elutriate and porewater bound considerably less (Table 3). A wide variation in the percent bound fraction of lake water with the filtration method occurred with pyrene ($62.4 \pm 31\%$; Table 3).

TABLE 1. COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR BENZO(*a*)PYRENE, USING A VARIETY OF RECOVERY METHODS

Method	Dosed Lake Water			Elutriate			Porewater		
	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
XAD Column	4.66	45.6	4.83	5.09	80.0	5.50	--**	--	--
	(1.05)	(20.1)*	to		(11.2)	to			
		n = 7	5.64		n = 6	7.56			
XAD Stirred	--	--	--	5.09	90.4	6.18	--	--	--
					n = 2	to			
						6.38			
Dialysis	--	--	--	11.33	64.4	5.05	--	--	--
				(3.64)	(6.5)	to			
					n = 5	5.38			
Centrifugation @ 20,000 *g*/Sep Pak	8.82	33.81	4.37	13.9	95.47	6.02	34.5	98.9	6.40
	(4.05)	(6.8)	to		(3.3)	to		n = 2	to
		n = 6	4.73		n = 3	6.99			6.41
Filtration/Sep Pak	5.93	29.94	3.75	5.09	62.43	4.72	12.45	79.77	5.18
	(1.02)	(10.8)	to		(23.7)	to		(11.0)	to
		n = 5	5.48		n = 12	6.73		n = 9	6.07

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment. Analysis of dosed lakewater was performed within 24 hours of preparation.
*± 1 S. D.
**Test not performed.

Centrifugation Mean recoveries for the centrifugation method were high and ranged from 82.8% (pyrene) to 100+% (*trans*-chlordane). Sorption of compounds to the stainless steel centrifuge tubes was lowest for endrin (7.33 ± 2.7%), and was only slightly higher for the other three compounds examined: BaP 15.11%, *trans*-chlordane 17.76%, and pyrene 18.02%. The centrifugation method showed the highest mean percentage of bound BaP in elutriate and porewater (Table 1) and the least *trans*-chlordane and pyrene binding in lakewater, among the various methods examined (Tables 2 - 3). For endrin, the percentage of bound contaminant with centrifugation was only about one third that of the filtration method in elutriate, and about half that of filtration in porewater (Table 3). For the most part, the range of partition coefficients obtained with the centrifugation method compared with those obtained with other methods. However, log K_p values were approximately 70 times higher for the centrifugation method, compared with filtration for *trans*-chlordane in porewater (Table 2).

B. Overall Methods Evaluation

No significant differences in the percent of bound BaP were determined among XAD, filtration, and centrifugation methods using two-way analysis of variance and post-hoc testing, although differences were seen among methods with the three other contaminants (Table 4). Significant differences in binding were seen among all aqueous phases contaminated with BaP, *trans*-chlordane, and endrin. Pyrene tended to partition similarly in Lake

Michigan water and elutriates even though the mean percent of bound pyrene differed between the partitioning methods examined (Tables 3 and 4). Overall, BaP showed the most consistant partitioning among the methods.

C. Contaminant Partitioning After Sediment Manipulation

There were no significant differences in the binding of BaP in porewater and elutriates produced from sediment aged 7 days versus 371 days (t = 0.961, 6 d.f.; Table 5), where almost all BaP was bound. Binding in porewater and elutriates produced from *trans*-chlordane and pyrene dosed sediments was variable and did not follow a linear trend with the amount of sediment aging or manipulation. Significant differences in the percent of bound contaminant were seen between elutriates and porewaters prepared from the same sediment containing *trans*-chlordane, pyrene, and endrin (*trans*-chlordane, t = 2.364, 11 d.f.; pyrene, t = 4.170, 18 d. f.; endrin, t = 6.180, 9 d.f.; Table 5). For all phases and components, the least percent of binding was found in elutriates and porewater produced from endrin dosed sediment. Only about 10% of endrin was bound in elutriates prepared from aged sediment, and stayed relatively constant with sediment manipulation. Binding of endrin in porewater was 2 to 3 times that of elutriate fractions, and changed significantly after sediment manipulation (Table 5).

TABLE 2. COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR *trans*-CHLORDANE, USING A VARIETY OF RECOVERY METHODS

	Dosed Lake Water			Elutriate			Porewater		
Method	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
XAD Column	4.66 (1.05)	47.8 (17.1)* n = 7	4.64 to 5.70	5.09	72.6 (11.0) n = 6	5.541 to 6.03	--**	--	--
XAD Stirred	--	--	--	5.09	60.4 n = 2	5.21 to 5.78	--	--	--
Centrifugation @ 20,000 *g*/Sep Pak	8.82 (4.05)	13.42 (3.1) n = 6	4.90 to 5.08	13.9	64.52 (3.4) n = 3	5.08 to 5.20	34.5	83.96 n = 2	6.89 to 6.98
Filtration/Sep Pak	6.42 (1.5)	40.34 (10.7) n = 5	4.82 to 5.38	5.09	60.20 (18.0) n = 13	4.88 to 6.18	13.34	73.28 (10.7) n = 7	4.97 to 5.80

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment. Analysis of dosed lakewater was performed within 24 hours of preparation.

* ± 1 S. D.

**Test not performed.

TABLE 3. COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR ENDRIN AND PYRENE, USING SEP PAK SEPARATION FOLLOWING CENTRIFUGATION AND FILTRATION METHODS

Method	Dosed Lake Water			Elutriate			Porewater		
	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
Endrin, Filtration	5.65 (2.5)*	69.51 (13.1) n = 11	4.54 to 5.79	11.34 (3.3)	37.93 (11.4) n = 9	3.25 to 4.91	21.8 (2.8)	54.65 (13.4) n = 10	4.41 to 5.14
Endrin, Centrifugation	--**	--	--	3.61 (0.3)	10.63 (0.7) n = 5	4.46 to 4.55	11.35 (0.9)	29.30 (6.7) n = 6	4.44 to 4.70
Pyrene, Filtration	7.08 (3.0)	62.43 (30.8) n = 15	3.96 to 6.80	11.34 (3.3)	58.85 (16.6) n = 10	4.79 to 5.68	21.8 (2.8)	65.14 (16.1) n = 11	4.55 to 5.64
Pyrene, Centrifugation	6.33	5.40 (0.8) n = 6	3.84 to 4.04	8.84 (9.1)	37.88 (6.4) n = 7	4.65 to 5.18	12.7 (2.4)	85.44 (4.9) n = 9	5.54 to 5.92

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment. Analysis of dosed lakewater was performed within 24 hours of preparation.

* ± 1 S. D.

**Test not performed.

TABLE 4. MULTIPLE COMPARISON RESULTS FOR DIFFERENCES AMONG MEAN FRACTIONS OF BOUND CONTAMINANTS OBTAINED IN PHASES OF AQUEOUS MEDIA AND METHODOLOGIES FOR THE CONTAMINANTS USED

	Benzo(*a*)pyrene			*trans*-Chlordane		
	XAD-4	Filtration	Centrifugation	XAD-4	Filtration	Centrifugation
Lake Michigan Water	1A	1A	1A	1A	1B	1A
Porewater	ND*	2A	2A	ND	2B	2A
Elutriate	3A	3A	3A	3A	3B	3A

	Pyrene		Endrin	
	Filtration	Centrifugation	Filtration	Centrifugation
Lake Michigan Water	1A	1B	1A	ND
Porewater	2A	2B	2A	2B
Elutriate	1A	1B	3A	3B

Means with same grouping letter for a method and same grouping numeral for an aqueous phase are not significantly different at P > 0.05, using Scheffe's multiple range test (*i. e.*, No significant differences were seen among methods that used BaP-dosed Lake Michigan water. However, significant differences were seen for BaP in Lake Michigan water, porewater, and elutriate among all methods).

*Comparison not done.

D. Partitioning Over Bioassay Testing Intervals

Because the driving force behind this work was evaluation of bioaccumulation data, the bioavailable fractions of contaminants in porewater and elutriates were followed over the time course of a typical bioassay. The bioavailable fractions in porewater and elutriates stayed relatively constant from 1 to 96 hours in bioassays using midge larvae (Table 6). The percent of freely dissolved BaP, *trans*-chlordane, and endrin was significantly different in porewater versus elutriates produced from the same sediment (BaP, t = -4.323, 10 d.f.; *trans*-chlordane, t = -3.023, 10 d.f.; endrin, t = -3.500, 8 d.f.), with elutriates consistently showing a larger bioavailable fraction (Table 6). The percent of freely dissolved pyrene was not significantly different in porewater and elutriates (t = -2.123, 10 d.f.), where approximately half of the pyrene was freely dissolved.

TABLE 5. COMPARISON OF CONTAMINANT BINDING IN ELUTRIATE AND POREWATER PRODUCED FROM SEDIMENTS PREVIOUSLY MANIPULATED

Compound	Sediment Age (days)	Time Between Last Mixing and Partitioning Study (days)	% Bound, Porewater	% Bound, Elutriate
Benzo(*a*)pyrene	371	126	100.00 n = 2	100.00 n = 2
Benzo(*a*)pyrene	7	7	99.25 (0.65)* n = 3	95.47 (3.3) n = 3
Pyrene	219	101	100.00 n = 2	100.00 n = 2
Pyrene	298	253	88.23 (2.7) n = 3	34.97 (1.6) n = 3
Pyrene	306**	8	79.28 (0.3) n = 3	33.10 n = 2
Pyrene	60	28	88.82 (2.0) n = 3	47.01 n = 2
trans-Chlordane	371	126	65.90 n = 1	41.70 n = 2
trans-Chlordane	60	28	96.73 (3.1) n = 3	89.52 n = 2
trans-Chlordane	7	7	83.96 n = 2	64.52 (3.4) n = 3
Endrin	298	253	34.91 (4.0) n = 3	10.74 (0.8) n = 3
Endrin	306	8	23.68 (0.9) n = 3	10.46 n = 2

All values were obtained via Sep Pak separation of freely dissolved and bound fractions after centrifugation at 20,000 g.

* ± 1 S. D.

** Sediment was used from previous (298 day old) assay, conducted 8 days previous.

TABLE 6. PARTITION COEFFICIENTS AND PERCENT FREELY DISSOLVED CONTAMINANTS IN ELUTRIATES AND POREWATER OVER THE TIME COURSE OF BIOACCUMULATION ASSAYS

Compound	Exposure Period (hours)	Porewater % Freely Dissolved	Porewater log K_p	Elutriate % Freely Dissolved	Elutriate log K_p
Benzo(*a*)pyrene	1	24	5.25	53	4.90
	6	27	5.18	41	5.11
	24	14	5.52	38	5.17
	48	8	5.79	28	5.36
	72	19	5.38	32	5.28
	96	23	5.28	48	4.99
trans-Chlordane	1	37	4.97	52	5.25
	6	36	5.00	52	5.25
	24	20	5.36	37	5.52
	48	13	5.59	37	5.52
	72	22	5.29	38	5.50
	96	25	5.23	81	4.65
Pyrene	1	53	4.56	64	4.79
	6	45	4.71	55	4.95
	24	54	4.55	50	5.05
	48	46	4.69	47	5.10
	72	48	4.66	58	4.91
	96	36	4.87	54	4.96
Endrin	1	62	4.41	79	3.25
	6	56	4.53	95	2.58
	24	62	4.41	72	3.43
	48	55	4.54	65	3.56
	72	49	4.65	72	3.41

All values were obtained via Sep Pak separation of freely dissolved and bound fractions after filtration through glass fiber filters.

DISCUSSION

From a practical standpoint, centrifugation and filtration methods yielded the most convenient techniques for determining partition coefficients in aqueous samples. Both methods took less time to perform than the XAD resin or dialysis methods. The centrifugation method produced the most consistent partition coefficients for each of the four contaminants tested. Partition coefficients obtained from the filtration method were less consistent, where individual samples differed by up to a factor of 692 for pyrene in dosed Lake Michigan water. The variation in partition coefficients may be due, in part, to differential sorption of the contaminants to the glass fiber filters and glassware used in the technique. Previous studies have used scintillation cocktail to rinse the filtering apparatus, with subsequent LSC (Eadie *et al.* 1990, 1992). These studies showed an average 4.3 to 9.2% of the total contaminant mass sorbed to the filtering apparatus. Such rinsing may have reduced the variability of the Kps that we obtained and lowered mean partition coefficients. Another factor contributing to the variation in Kp values may be the inability to determine exact TOC concentrations in individual samples. Total organic carbon samples were taken from representative samples of the aqueous fractions studied. The TOC concentration from XAD and filtration/Sep-Pak methods reported in Tables 1 and 2 was lower than that of the centrifuged supernatant from the centrifugation/Sep-Pak method. The variation in TOC between methodologies was probably due to sediment differences (aliquots of sediment were obtained over a period of several months) or aqueous TOC composition over the course of the study. Sample to sample variation of TOC may have resulted in an overestimation or underestimation of the calculated partition coefficients.

Fewer surfaces were available for contaminant sorption in the centrifugation method, compared to the filtration method. Sorption to the stainless steel centrifuge tubes averaged less than the 40-63% previously reported for fluoranthene and DDE (Schults *et al.* 1992). Centrifuging the samples at 20,000*g* may have lessened the chance for colloids and other microparticulates to be included in the freely dissolved fractions of samples. This may have contributed to the narrower range of Kp values obtained with the centrifugation method.

Partition coefficients obtained from the filtration method compare favorably with previous results of BaP and pyrene in Lake Michigan water (Landrum *et al.* 1985, 1987). In a study that compared filtration/SepPak techniques with dialysis, partition coefficients were significantly higher using dialysis (Landrum *et al.* 1984). Although the partitioning of only one compound (BaP) in elutriate fractions could be compared using filtration and dialysis techniques in this study, Kp values were comparable for both methods.

The low recovery obtained with dialysis due to sorption onto surfaces may have been reduced by using dialysis membranes composed of a different material. Reliable results have been achieved when Spectra/Por 6 dialysis tubing was used (Carter and Suffet 1982, McCarthy and Jimenez 1985). The dialysis tubing used in the present study was composed of a cellulose ester that may have sorbed the contaminants more readily than the Spectra/Por 6 material made of regenerated natural cellulose. A study designed to compare sorption differences between the two types of dialysis tubing would test this idea.

The fact that partitioning did not significantly change from 1 to 96 hours in bioassays indicates that the bioavailability of the contaminants stayed relatively constant over the course of the exposures. Previous studies that examined partitioning between porewater and sediment particles in laboratory-dosed Lake Michigan sediment found partitioning of pyrene and phenanthrene to increase with contact time between 3 and 150 days (Landrum *et al.* 1992 *b*). In those studies, amphipods exposed to aged whole sediment experienced a loss of bioavailable contaminants with sediments aged up to 60 days, reflected in lowered uptake rate coefficients. Such results may depend on the conditions of the experiment (test duration of 96 h in this study was relatively short), the contaminant, and the indicator species used.

Pyrene was the only contaminant that did not partition differently between porewater and elutriates generated from the same sediment. Pyrene was also the least hydrophobic of the contaminants used. The differences in the percentages of freely dissolved contaminants between porewater and elutriate fractions for the three other contaminants suggests that elutriates may not bind these contaminants to as large an extent as porewater. Certainly, the length of contact time between the aqueous phase and sediment particles is shorter with prepared elutriates than with porewater generated from dosed sediment. However, it is possible that more soluble compounds, such as pyrene, equilibrate in aqueous phases more quickly than do less soluble compounds.

Significant differences in TOC composition among the aqueous fractions may have contributed to the differences in partition coefficients obtained for a single contaminant. Although contaminant concentrations in elutriates, porewater, and lakewater were normalized for the quantity of TOC in the samples (equation 3), the quality of TOC contained in dissolved organic material (DOC) most likely differed among samples. Previous studies showed that partitioning of organics such as BaP and napthalene is proportional to the hydrogen/carbon ratio of DOM in natural waters (Kukkonen 1991). Furthermore, the proportion of hydrophobic acids in DOM significantly correlates with contaminant partitioning (Kukkonen and Oikari 1991). Variation in the proportions of hydrophobic acids, hydrogen/carbon composition, and the amount of microparticulates and colloids were most likely responsible for both the relatively wide range of K_p values obtained among replicates of a single media and the significant differences in K_p values among porewater, elutriate, and lake water obtained for a single contaminant. Of the four methods studied, we obtained the greatest precision from reverse-phase column separation after centrifugation at 20,000*g*. This technique evidently excluded colloids and microparticulates, and was the most successful method for obtaining the most accurate measure of the "freely dissolved" phase of the contaminants in aqueous media.

We suggest that partitioning of contaminants in aqueous fractions after sediment manipulation tends to be compound dependent. BaP in aqueous fractions obtained from newly dosed and aged sediment was almost all bound. However, a threefold difference was seen in the amount of binding among elutriates obtained from pyrene-dosed sediment, and binding did not tend to reflect the duration of the contaminant-sediment interaction. Our data show that the researcher should be aware of changes in the bioavailability of contaminants in fractions obtained from whole sediment when designing and analyzing the results of toxicokinetic studies.

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